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the documents annexed hereto are true copies of:

Application forms P.1, P.2, provisional specification and drawings of South African Patent Application No. 2002/9250 as originally filed in the Republic of South Africa on 14 November 2002 in the name of POPPAUBREY CLOSE CORPORATION for an invention entitled:

" A MIXED ANIMAL FEED ".

Geteken te **PRETORIA** in die Republiek van Suid-Afrika, hierdie 6th dag van **February 2004**
Signed at in the Republic of South Africa, this day of

Registrar of Patents

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FORM P2

REPUBLIC OF SOUTH AFRICA

REGISTER OF PATENTS

PATENTS ACT, 1978

21	01 Official Application No. 2002/9250	22	Lodging Date : Provisional 2002 -11- 14	47	Acceptance Date
51	International Classification	23	Lodging Date : Complete		Granted Date

71 Full name(s)of Applicant(s)/Patentee(s)

POPPAUBREY CLOSE CORPORATION

71	Applicants substituted:	Date registered
71	Assignee(s)	Date registered

72 Full name(s)of Inventor(s)

DICKS, Leon Milner Theodore

Priority claimed	33	Country	31	Number	32	Date

54 Title of Invention

A MIXED ANIMAL FEED

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74 Address for service

JAN S DE VILLIERS, 1st FLOOR ECCLESIA BUILDING, 71 PLEIN STREET
(DOCEX 10) STELLENBOSCH, 7600 SOUTH AFRICA

61 Patent of Addition No.	Date of any change
Fresh Application based on	Date of any change

FORM P1

REPUBLIC OF SOUTH AFRICA

PATENTS ACT, 1978

APPLICATION FOR A PATENT AND ACKNOWLEDGEMENT OF RECEIPT

(Section 30(1) - Regulation 39)

The grant of a Patent is hereby requested by the undermentioned applicant(s) on the present application filed in duplicate

21	01	Official Application No.	22	Lodging Date	47	Applicant's Reference No:
		2002/9250		2002 -11- 14		H30-001P0214

71 Full name(s) of applicant(s)

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54 Title of invention

A MIXED ANIMAL FEED

The applicant claims priority as set out in the accompanying form P2
The earliest priority is
This application is for a Patent of Addition to Patent (Application) No.
This application is a fresh application in terms of S 37 and based on Application No.

21	01
21	01

This application is accompanied by:-

X	1a	A single copy of a provisional specification of	19 pages
	1b	Two copies of a complete specification of	pages
	2a	Informal drawings of	Nil sheets
	2b	Formal drawings of	sheets
	3	Publication particulars and abstract (form P8 in duplicate)	
	4	A copy of Figure of the drawings for the abstract	
	5	Assignment of invention (from the inventor(s)) or other evidence of title	
	6	Certified priority documents (documents)	
	7	Translation of priority documents (documents)	
	8	Assignment of priority rights	
	9	A copy of form P2 and the specification of S.A. Patent Application No.	21 01
	10	A declaration and power of attorney on form P3	
	11	Request for ante-dating on form P4	
	12	Request for classification on form P9	
	13a	Request for delay of acceptance on form P4	
	13b		

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Date 12th November, 2002

for the applicant

The duplicate will be returned to the applicant's address for service as proof of lodging but is not valid unless endorsed with official stamp

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FORM P6

REPUBLIC OF SOUTH AFRICA

PATENTS ACT, 1978

PROVISIONAL SPECIFICATION

Section 30 (1) — Regulation 27

21	01	Official application No.	22	Lodging date
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71 Full name(s) of applicant(s)

POPPAUBREY CLOSE CORPORATION

72 Full name(s) of inventor(s)

DICKS, Leon Milner Theodore

54 Title of invention

A MIXED ANIMAL FEED

A MIXED ANIMAL FEED

FIELD OF THE INVENTION

This invention relates to a mixed animal feed in which there is utilized an agricultural byproduct. More particularly, but not exclusively, the invention relates to an animal feed that is suitable for use in feeding sheep.

BACKGROUND TO THE INVENTION

Wheat is the dominant forage available during summer in sheep farming areas that are subject to a Mediterranean climate, and of particular interest, in South Africa. Stubble, however, is reported to have low levels of nitrogen and available carbohydrates, a high cell wall content and poor digestibility (Dann and Coombe, 1987), rendering it unsuitable to meet the high nutrient requirements of producing sheep (Aitchinson, 1988). It is therefore commonplace to provide supplementary feeding especially for ewes grazing stubble to provide additional energy and protein (Aitchinson, 1998; Brand, 1997a). One common form of supplementary feed is lucerne hay.

Grapes are widely grown in the Mediterranean area, producing considerable quantities of by-products in the form of grape seeds and husks resulting from the fruit juice and wine producing industries. Traditionally, the grape seeds and husks are dumped or used as compost.

It has now surprisingly been found that a useful mixed animal feed can be produced using this agricultural by-product.

OBJECT OF THE INVENTION

It is, accordingly, an object of the invention to provide a mixed animal feed that embodies a proportion of agricultural by-product material.

SUMMARY OF THE INVENTION

In accordance with this invention there is provided a mixed animal feed comprising a conventional grass based feed admixed with up to 60% by weight of by-product grape seed optionally mixed with grape husks.

Further features of the invention provide for the conventional grass based feed to be lucerne hay, typically in pelleted or other agglomerated form; for the grape seed and, where present, grape husks, to be pretreated with at least one, and typically a combination of tannin degrading bacteria, preferably tannin-hydrolyzing lactic acid bacteria; for the grape seeds and any grape husks admixed therewith to be dried, milled, typically in a hammer mill and pelleted for admixture with the conventional grass based feed; and for the milled by-product, in instances in which it is to be pretreated with bacteria, to be suspended in a suspension that is inoculated with the bacteria prior to drying and pelletising.

Regarding suitable bacteria to be employed for the aforesaid purpose, a number of bacteria capable of degrading tannins have been identified, viz. Streptococcus bovis, Streptococcus caprinus and Streptococcus gallolyticus (Brooker et al., 1994; Ossawa et al., 1995; Sly et al., 1997). Many of the strains were isolated from the rumen of goats browsing on Acacia (rich in tannins). The strains were resistant to condensed tannins from Acacia anuera and grew in media containing concentrations as high as 2.5%, w/v (Brooker et al., 1994).

Despite the identification of diverse populations of tannin tolerant bacteria from a number of animals, e.g. goat (Brooker et al., 1994; McSweeny et al., 1996), koalas (Osawa, 1990; 1992; Osawa and Sly, 1992) and other ruminants (Nelson et al., 1995; Odenyo and Osuji, 1998), little is known about the relationships these organisms have with other (normal) gut microflora and the mechanisms they use to degrade tannins (Brooker, 2000).

The invention is therefore based on the fact that the basal diet of sheep, consisting of lucerne hay, can be altered by replacing various proportions, and up to about one half (50%) of the lucerne hay, with grape seeds and husks. The invention, in its preferred implementation is still further based on the fact that binding of tannins (from the grape seeds and husks) to proteins can be decreased by treating the grape seeds and husks beforehand with a combination of tannin-hydrolyzing lactic acid bacteria.

In order that the invention may be more fully understood an extended description thereof and the results of various investigations that have been carried out to date, follow.

DETAILED DESCRIPTION OF VARIOUS ASPECTS OF THE INVENTION

A breakdown of the protein, fat and various fibre contents of the seeds, and a combination of husks and seeds, of a mixture of four red wine cultivars (Merlot, Shiraz, Carignan and Cabernet Sauvignon), is presented in Table 1. The amino acid composition of the latter is presented in Table 2. In each instance comparisons were made with plant products that are normally added to animal feed.

The overall chemical composition of grape pips and a combination of husks and pips was very similar (Table 1). The protein content of the husks and pips was lower than that recorded for Alfalfa (12.7% versus 15%). However, the fat content of the grape pips was much higher compared to Alfalfa (10.3

and 7.9% versus 1.6%). The higher fat content would result in an increase in energy production and is, in the light of this, considered to be an advantage. The higher ADF (acid detergent fiber) and NDF (neutral detergent fiber) contents recorded in grape pips and husks would, however, slow down the enzymatic conversion of the animal feed in the gut and is thus considered to be a disadvantage.

The grape seeds contained a larger variety of amino acids than was recorded for maize and soya (Table 2), rendering it a more suitable animal feed. However, Lysine, a limiting and very important amino acid in animal feed, is present at very low concentrations (0.387%). This indicated that a mixed feed could work effectively.

Tannins are not easily degradable. Metabolic energy in animal feed is derived mostly from starches, sugars, carbohydrates, fats and oils. Binding of tannins to any of the latter substrates is believed to restrict the digestibility of the substrate (Tangendjaja, 2000), which in turn may lead to a lowering in the digestibility of the substrates in an animal feed. Furthermore, binding of tannins to proteins is believed to the produce insoluble or soluble tannin-protein (and also tannin-enzyme) complexes which, when ingested, may lead to a lowering of enzyme activity, followed by a decrease in intestinal metabolic activity which may lead to malnutrition.

Tannins occur in red grapes, and are present either in hydrolyzable or condensed forms (Butler, 1989). There is an inverse relationship between high tannin level in forage and palatability, digestibility and voluntary intake. Grape seeds (pips), -husks and -skins are rich in condensed tannin content (approximately 14g STE, sorghum Tannin Equivalents, per kg dry mass).

In the light of this it was determined that the basal diet of sheep, consisting of lucerne hay, can be altered by replacing up to one half (50%) of the lucerne hay with grape seeds and husks.

Grape seeds and husks of Merlot, Shiraz, Carignan, Cabernet Sauvignon were dried, pooled in equal amounts by weight, mixed and pelleted. The basal diet, pelleted lucerne hay, was then supplemented for test purposes with the grape seeds and husks such the latter contributed 0, 12.5, 25.0, 37.5 and 50.0 % of the total dry matter intake.

Twenty Dohne merino ram lambs (41.4 ± 2.3 kg) were used in a voluntary intake and digestion trail. A completely randomized design was used and the animals were assigned to four diets consisting of 0, 12.5, 25.0, 37.5 and 50 % grape seeds and husks.

The chemical composition of the five diets, and the grape seeds and husks, before being fed to the animals, is listed in Table 3. The protein content decreased as the percentage grape seeds and husks increased in the diet, while the CP-ADF (protein attached to cell walls, and therefore indigestible) increased. The level of condensed tannin increased dramatically as the percentage grape seeds and husks increased in the diets. The condensed tannins are reported to bind to proteins and sometimes may reduce the protein digestibility (Walton et al., 2001).

All the animals were vaccinated and drenched before the experiments, and were kept in individual pens. Feeding was ad lib and at a level close to maintenance (40-45 g DM $kg^{-1}LW^{0.75}$ per day), as recommended by Van Es and Van der Meer (1980). During the trail, which lasted 35 days (14 days for acclimatization and 21 days for the experiment), daily water and dry matter intake were measured. Faeces were collected daily from each animal, dried at 50°C for 96h, and ground through a 1 mm screen.

The fecal, orts and feed samples were analyzed for dry matter (DM), ash, crude protein (CP) and ether extract (EE) according to AOAC (1984) methods. To determine neutral detergent fiber (NDF) and acid detergent fiber (ADF) the methods proposed by Van Soest et al. (1991) were followed. Acid-detergent insoluble nitrogen (ADIN) was measured (Licitra et al., 1996), and the results reported as crude protein (ADF-CP). The sorghum tannin equivalent method was used for determination of condensed tannins.

Blood samples (10 ml) were taken from each sheep at the end of the digestibility trail. Blood was taken from the jugular vein into heparinized tubes and centrifuged for 20 min at 3 000 rpm (revolutions per minute) o separate the plasma, which was stored at -20°C. The plasma was analyzed according to normal procedures used for diagnosing domestic animal hepatic and kidney damage and general disorders (Kaneko, 1989). Components measured were total protein, plasma urea nitrogen and creatinine. In addition, the plasma enzymes aspartate aminotransferase (AST) and gamma glutamyltranspeptidase (GGT) were measured.

The average feed intake, water intake and blood metabolic profile data are listed in Table 4.

According to Table 4 the voluntary feed and water intake were not significantly influenced ($P \geq 0.05$) by the percentage grape seeds and husks included in the diet. The final body weight was also not negatively influenced ($P \geq 0.05$) by the inclusion of grape seeds (pips) and husks up to 50% of the diet. The presence of tannin in a forage has been assumed to affect voluntary intake (McLeod, 1974). However, in this trial intake problems were not observed with inclusion levels of up to 50 % of diet dry matter.

There were no differences between diets in any plasma metabolite, except for blood urea nitrogen (Table 4). An increase of creatinine can be related

with renal failure, but the level found in the present study fell within the normal range for sheep (Kaneko, 1989). No significant changes in plasma enzymes AST and GGT were found. These enzymes are used to detect if tannin-related hepatotoxicity occurred (Zhu & Filippish, 1992). In sheep fed with lucerne hay, the blood urea nitrogen concentration was higher ($P \leq 0.05$) than in sheep fed with the diets including the grape seeds and husks, which is directly related to the limitation in protein digestibility in sheep fed husks and pips. Similar results were found by Silanikove et al. (1996) where urea concentration was higher in goats fed with tannin-rich leaves than when fed wheat straw.

The digestibility of the five diets is shown in Table 5.

According to this data, grape seeds and husks could be considered low quality roughage. The digestibility of the crude protein, neutral detergent fiber and acid detergent fiber decreased significantly, while the dry matter digestibility showed a strong tendency towards a lower digestibility as the percentage grape seeds and husks increased in the diet. This result may be due to several factors. Firstly, the husks and pips had a much higher level of CP-ADF (crude protein bound to the indigestible fiber fraction) than that of lucerne hay (71.5 % vs. 17.6 % of the total protein is bound to the fiber). Secondly, it could be due to the presence of condensed tannins in husks and pips. The condensed tannin content of the husks and pips was 20 times higher than that of lucerne hay. These compounds may form complexes with proteins and carbohydrates (Makkar et al., 1996), decreasing the available protein and energy for rumen microorganisms.

The decrease in diet digestibility as the percentage husks and pips included in the diet increased, might be due to factors such as high levels of proteins bound to the acid detergent fiber and condensed tannins. However, sheep accepted an inclusion up to 50 % in the diet and toxic effects were not evident in this study.

It is therefore not exactly clear as to whether or not the effects of the tannins will adversely affect the mixed feed according to the invention but, nevertheless, an attempt was made to diminish very effects utilizing tannin-hydrolyzing lactic acid bacteria.

Isolation of tannin hydrolyzing strains:

Tannin-hydrolyzing lactic acid bacteria were isolated from the faeces of goats and sheep. Fecal samples were streaked onto MRS Agar (Merck) plates, incubated for 3-5 days at 37°C, and colonies of various morphology selected. A total of 200 isolates were collected and tested for tannin hydrolysis as follows:

BHI (Brain Heart Infusion) Agar (Merck), supplemented with 0.5% yeast extract (Unilab), was overlaid with 5 ml of a 2% (w/v) tannic acid (Unilab, 5944000) solution and left at room temperature (approx. 25°C) for at least one hour. The excess tannic acid solution was then decanted and rinsed from the plates by using sterile distilled water. The plates were left to dry and then inoculated with 100µl of an active growing culture from BHI broth. The plates were incubated at 37°C for at least 24h.

From the above plates, six colonies tested positive for the degradation of tannic acid (observed as clearing zones surrounding the colonies). Pure cultures were obtained by repeated streaking onto BHI Agar and stores at -80°C in 40% (v/v) glycerol. Four strains with the highest tannin hydrolysis activity, based on the reactions recorded on the BHI Agar plates, were selected.

Identification and characteristics of the strains:

Identification was done by using the API 50CHL carbohydrate fermentation profile test system. Two of the strains were identified as Streptococcus spp.

and two as Lactobacillus spp. The strains were numbered TS1 and TS2 (streptococci) and TL1 and TL2 (lactobacilli).

Characteristics of the strains:

Gram-positive, catalase negative. Cocci in chains (streptococci) or elongated cells (lactobacilli).
None of the strains could utilize tannic acid as a sole carbon source.
L-lactic acid is produced from the fermentation of glucose.
Gucose, starch, cellobiose, gallactose, mannose, trehalose, sucrose, lactose, fructose, maltose, raffinose and inulin are fermented. Rhamnose, glycerol, xylose, sorbitol, inositol and arabinose are not fermented.
Optimal growth at 37°C.
Good growth in the absence of CO_2.
Growth in MRS broth, but prefers BHI broth.

Treatment of the grape seeds and husks:

The dried grape seeds and husks were milled in a hammer mill to a particle size of 1mm in diameter. Three parts of sterile distilled water were added to one part of the milled grape seeds. Peptone (2%, w/w) was added to the grape seed suspension and then heat-treated for 2 min at 100°C. The heated suspension was left to cool down to room temperature (approx. 25°C). One of the batches was inoculated with 10% (v/v) of an equal combination of strains TS1, TS2, TL1 and TL2. The other batch was inoculated with a control Lactobacillus sp. that could grow in the suspension, but tested slight positive for tannin hydrolysis.

Treatment of the grape seed (pip) and husk suspension with tannin-hydrolyzing bacteria resulted in the hydrolysis ("splitting") of tannins from the protein (peptone added to the suspension). A clear increase in free tannins, as determined with a standard acid butanol and spectrophotometric assay,

was recorded in the batch treated with the tannin-hydrolyzing bacteria (Fig. 1). The highest level of free tannins (OD = 0.2835) was recorded after 11 days of treatment with the tannin-hydrolyzing bacteria. The control batch revealed much lower hydrolytic activity (Fig. 1).

Binding of tannins to proteins can be decreased by treating the grape seeds and husks beforehand with a combination of tannin-hydrolyzing lactic acid bacteria (TS1, TS2, TL1 and TL2).

In vitro tests are currently being done to determine the digestibility of the treated grape seed (pip) and husk suspension. The method described by Tilley et al. (1963) will be used.

REFERENCES

Aitchison, E., 1988. Cereal straw and stubble as sheep feed. J. Agric. W. Aust. 29: 96-101.

AOAC, 1984. Official Methods and Analysis, 15th Edition. Association of Official Analytical Chemists, Washington, DC, pp. 1141.

Australian Centre for International Agricultural Research.

Brand, T.S., Franck, F., Durand, A. & Coetzee, J., 1997. Use of varying combinations of energy and protein sources as supplementary feed for lambing ewes grazing cereal stubble. Aust. J. Exp. Agric. 37: 1-9.

Brooker, J.D. 2000. International Workshop on Tannins in Livestock and Human Nutrition.
http://www.aciar.gov.au/publications/proceedings/92/index.htm

Brooker, J.D., 1994 Streptococcus caprinus sp.nov. a tannin-resistant ruminal bacterium from feral goats. Letts. Appl. Microbiol. 18:313-318.

Butler, L.G., 1989. Sorghum polyphenols. In: Cheeke, P.R. (Ed.), Toxicants of Plant Origin, Vol. 4. Phenolics, CRC Press, Boca Raton, FL, pp. 95-114.

Dann, P.R. & Coombe, J.B., 1987. Utilization of fodder crops and crop residues. In: Temperate Pastures, their production, use and management. Eds. Wheeler, L.W., Pearson, C.G. and Robards, G.E., Australian Wool Corporation, Australia, p.517-525.

Flores, M.P., Castanon, J.I.L. & Mcnab, J.M., 1994. Effect of tannins on starch digestibility and TMEn of triticale and semi-purified starches from triticale and field beans. Br. Poult. Sci. 35: 281-286.

Kaneko, J.J., 1989. Clinical Biochemistry of Domestic Animals, 4th Edition. Academic Press, New York, pp. 932.

Licitra, G., Hernández, T.M. & Van Soest, P.J., 1996. Standardization of procedures for nitrogen fractionation of ruminal feeds. Anim. Feed Sci. Technol. 57: 347-358.

Makkar, H.P.S., Goodchild, A.V. & Abd El-Moneim, A.M., 1996. Cell-constituents, tanning levels by chemical biological assays and nutritional value of some legume foliage and straws. . Sci. Food Agric. 71: 129-136.

McLeod, M.N., 1974. Plant tannins – their role in forage quality. Nutr. Abst. Rev. 44: 803-815.

McSweeny, C.S. 1998. Isolation and characterisation of proteolytic ruminal bacteria from sheep and goats fed the tannin-containing shrub legume Calliandra calothyrsus. Appl. Environ. Microbiol. 65: 3075-3083.

Nelson, K.E. 1995. Isolation and characterisation of an anaerobic ruminal bacterium capable of degrading hydrolysable tannins. Appl. Environ. Microbiol. 61:3293-3298.

Odenyo, A.A., Osuji, P.O. 1998. Tannin-tolerant ruminal bacteria from East African ruminants. Can. J. Microbiol. 44:905-909.

Osawa, R. 1990. Formation of a clear zone on tannin-treated brain heart infusion agar by a Streptococcus sp. isolated from faeces of koalas. Appl. Environ. Microbiol. 56:829-831.

Osawa, R., Fujisawa, T., Sly, L.I. 1995. Streptococcus gallolyticus sp. nov., gallate-degrading organisms formerly assigned to Streptococcus bovis. Syst. Appl. Microbiol. 18:74-78.

Osawa, R., Sly, L.I. 1992. Occurrence of tannin-protein complex degrading Streptococcus sp. in faeces of various animals. Syst. Appl. Microbiol. 15:144-147.

Silanikove, N., Gilboa, N., Perevolotsky, A. & Nisan, Z., 1996. Goats fed tannin-containing leaves do not exhibit toxic syndromes. Small Rumin. Res. 21: pp.195-201.

Sly, L.I., Cahill, M.M., Osawa, R., Fujisawa, T. 1997. The tannin-degrading species Streptococcus gallolyticus and Streptococcus caprinus are subjective synonyms. Int. J. Syst. Bacteriol. 47:893-894.

Tangendjaja,-B. 2000. Tannins and Ruminant Production in Indonesia. Australian Centre for International Agricultural Research. http://www.aciar.gov.au/publications/proceedings/92/index.htm

Tilley. J.M.A. & Terry, R.A., 1963. A two-stage technique for the in vitro digestion of forage crops. . Br. Grassl. Soc. 18: 104-111.

Van Es, A.J.H., Van der Meer, J.M., 1980. Methods of analysis for predicting the energy and protein value of feeds for farm animals. In: Proceeding of the 31st Annual Meeting, EAAP, Netherlands. Pp. 39-43.

Van Soest, P.J., Robertson, J.B. & Lewis, B.A., 1991. Methods for dietary fiber, neutral detergent fiber and nonstarch polysaccharides in relation to animal nutrition. . Dairy. Sci. 74: 3583-3597.

Walton, J.P., Waghorn, G.C., Plaizer, J.C., Birtles, M. & McBride, B.W., 2001. Influence of condensed tannins on gut morphology in sheep fed Lotus peduncultus. Can. J. Anim. Sci. 81: 605-607.

Zhu, J. & Filippish, L.J., 1992. Tannic acid intoxication in sheep and mice. Res. Vet. Sci. 63: 280-292.

Table 1: Chemical composition of unpressed and pressed grape seeds (pips)

Chemical component (%)	**Grape pips**	**Husks and pips**	**Alfalfa**
ADF	52.4	40.8	35
NDF	58.2	42.7	38.5
DM	90.8	94	89
ASH	2.3	-	7.9
Protein	8.2	12.7	15
Fat	10.3	7.9	1.6
Fiber	35.7	21.9	28

Table 2: Amino acid content (%) of the grape seeds (pips)

Amino acid	Grape pips	Maize	Soya
Amino Acid Rec	8.656		
Aspartic acid	0.758		
Threonine	0.225	2.13	1.82
Serine	0.365		
Glutamic acid	2.040		
Proline	0.564		
Glycine	0.840		
Alanine	0.360		
Valine	0.415	1	2.36
Methionine	0.034	1.51	0.63
Isoleucine	0.355	2.72	2.28
Leucine	0.565	10.38	3.55
Tyrosine	0.181		
Pheynylalanine	0.337	3.94	2.36
Histidine	0.226	1.3	1.23
Lysine	0.387	1.07	2.89
Arginine	0.546	2	3.45
Ammonia	0.680		

Table 3: **Physical (on an air dry basis) composition (%) and chemical (on a dry matter basis) composition (%) of the experimental diets**

Experimental diet	1	2	3	4	5	Grape pips & husks
Lucerne hay	100	87.5	75.0	62.5	50	
Grape pips and husks	0	12.5	25.0	37.5	50	
Chemical composition						
Dry matter	92.5	92.1	92.0	92.0	92.4	92.3
Organic matter	88.9	89.4	89.1	90.7	92.0	92.9
Ash	11.1	10.6	10.9	9.3	8.0	7.1
Crude protein	18.2	17.9	17.2	16.2	15.1	13.7
Neutral detergent fibre	44.0	43.4	43.8	43.9	43.6	43.3
Acid detergent fibre	33.4	34.5	36.3	37.0	38.5	43.4
CP-ADF (g/100 g CP[1])	3.2	4.3	4.8	5.7	5.5	9.8
Ether extract (fat)	2.4	3.4	4.6	6.9	7.2	11.0
Total condensed tannins, gSTE[2]/kg DM	0.7	2.4	4.1	5.7	7.4	14.1

[1] CP = Crude protein

[2] STE = Sorghum Tannin Equivalents

Table 4: Average feed, water intake and blood metabolic profile of sheep fed the different diets

Item	Lucerne hay: Grape seeds (pips) and husks						
	100:0	87.5:12.5	75:25	62.5:37.5	50:50	SEM	P
Initial body weight, kg	41.7	41.4	41.4	41.5	40.8	2.25	0.9
Final body weight, kg	45.0	44.6	43.3	43.8	41.5	2.40	0.8
DM intake, g/day	1840	1916	1881	1943	1952	148.5	0.9
DM intake/$W^{0.75}$, g/day	110	116	114	117	119	7.08	0.9
Water intake, ℓ/day	7.74	7.49	7.67	6.84	6.19	0.77	0.8
Water intake/W^{075}, ℓ/day							0.4
Blood urea nitrogen, mg/100 ml	9.6^{a}	7.4^{b}	7.5^{b}	6.4^{b}	6.7^{b}	0.73	0.0
Total protein, mg/100 ml	68.0	67.5	69.8	71.3	68.3	2.36	0.7
Creatinine, mg/100 ml	118.8	122.0	119.3	125.3	124.8	5.50	0.8
AST, units/ℓ	75.8	93.3	73.5	80.5	89.8	6.25	0.2
GGT, units/ℓ	80.5	70.0	72.0	76.0	68.5	4.26	0.3

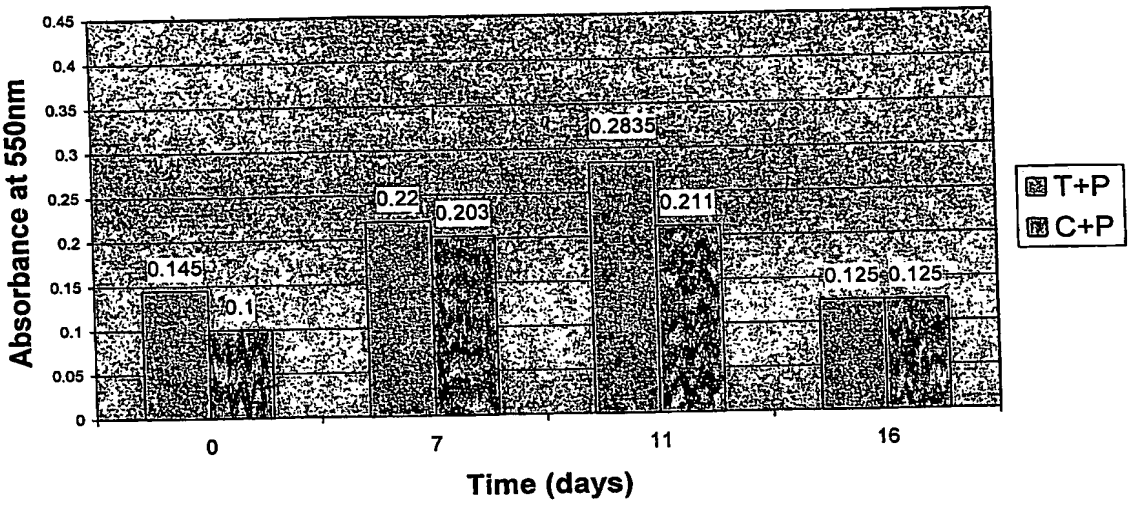
^{a,b,c} Values in rows bearing different superscript letters shows significant ($P \leq 0.05$) differences

Table 5: Apparent digestion coefficients of the diets

Item	Lucerne hay: Grape seeds (pips) and husks						
	100:0	87.5:12.5	75:25	62.5:37.5	50:50	SEM	P
Apparent digestibility (%)							
Dry matter	57.1	53.9	50.4	46.8	48.0	2.45	0.053
Crude protein	68.4^{a}	64.2^{ab}	59.0^{b}	56.3^{bc}	52.5^{c}	1.91	0.0002
Neutral detergent fibre	42.6^{a}	33.5^{b}	32.3^{b}	23.9^{c}	18.5^{c}	2.12	<0.000
Acid detergent fibre	39.9^{a}	26.4^{b}	23.7^{b}c	17.1^{cd}	12.8^{d}	3.21	0.0003
Ether extract (fat)	39.3^{a}	66.1^{b}	77.7^{c}	82.6^{c}	79.0^{c}	3.82	<0.000

^{a,b,c} Values in rows bearing different superscript letters shows significant ($P \leq 0.05$) differences

Fig. 1. The hydrolysis ("splitting") of tannins from peptone in a suspension containing grape seeds (pips) and husks.
T+P = tannin hydrolyxing bacteria added, C+P = control organism added.



Whilst it is not yet clear as to the long-term effects of utilizing grape seeds and husks that has not been treated to relieve the effects of the height tannin content as a part of an animal feed typically containing as the other part lucerne hay it is expected that the treated great seeds and husks will in any event be highly useful as a part of such feed and will put to good use an agricultural byproduct that does not find any particular present use.

Dated this 12th day of November 2002

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for the applicant